Effect of protein source and quantity on protein metabolism in elderly women.

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ABSTRACT To study sequentially the effect of meal feeding and the effect of protein source and quantity on whole-body protein metabolism, 12 elderly women consumed 3 diets differing in both the quantity and source of protein (diet A: 5.3% of energy intake provided by animal protein and 5.0% by vegetable protein; diet B: 14.5% of energy provided by animal protein and 5.1% by vegetable protein; diet C: 5.0% of energy provided by animal protein and 15.1% by vegetable protein). The diets were consumed for 2 wk with a 2-wk interval between diets. At the end of each dietary period, nitrogen balance and protein turnover were measured. Protein turnover was measured during 4 h of fasting followed by 4 h of feeding. Comparisons were made between fasted and fed periods (within one diet) and between the diets to study the effect of the protein source and quantity. Mean nitrogen balance did not differ significantly from zero during diets B and C and was not affected by the protein source. Meal feeding resulted in increased protein flux and protein oxidation and decreased protein breakdown compared with the postabsorptive values; there was no effect of feeding on protein synthesis. With the high-vegetable-protein diet, protein breakdown in the absorptive state was not inhibited to the same extent as during the high-animal-protein diet, resulting in less net protein synthesis during the high-vegetable-protein diet than during the high-animal-protein diet. *Am J Clin Nutr* 1998;68:1228–35.

KEY WORDS Protein turnover, elderly women, protein source, animal protein, vegetable protein, protein metabolism

INTRODUCTION

Human subjects are able to maintain nitrogen balance over a wide range of dietary protein intakes. This raises the question not only of what is the minimal requirement for dietary protein but also what is the optimal intake. The nitrogen balance technique gives no information about this optimum because it simply reflects the balance between whole-body protein synthesis and breakdown. Balance can be achieved within a wide range of protein turnover rates. Therefore, data on how rates of body protein synthesis and breakdown are affected by dietary intake represent an important step in understanding the metabolic significance of differences in dietary intake. Previously, several studies were performed to explore the influence of dietary protein on whole-body protein turnover in young adults as reviewed by Garlick et al (1). It was concluded that an increase in dietary protein affects protein turnover at 2 levels. First, there is an immediate response to the increased intake of protein in meals that consists almost exclusively of a reduction in the magnitude of the protein breakdown rate. Second, there is a long-term adaptation (1–2 wk) that involves an increase in the basal (postabsorptive) rates of both synthesis and degradation, eg, an increase in whole-body protein turnover.

Only a few studies have been undertaken in the elderly (2–5) describing protein turnover as measured during one level of protein intake. These studies were conducted to study the effect of aging on whole-body protein turnover. Recently, our group investigated the effect of different protein intakes (protein as 12% and 21% of total energy intake) on nitrogen balance and protein turnover (as measured with [^{15}N]glycine after an overnight fast) in young and elderly subjects (6, 7). From these studies it was concluded that protein turnover was significantly higher during the 21%-protein diet than during the 12%-protein diet in both elderly and young subjects. During the 12%-protein diet, young adults had higher protein turnover rates than did elderly subjects. During the higher protein intake (21% protein), protein turnover of young men was comparable with that of elderly men whereas young women had still higher protein turnover rates than elderly women (even when corrections were made for differences in body composition).

The aim of the present study, therefore, was 2-fold. First, we investigated whether an increase in protein intake would increase protein turnover in both the fasted and fed states. Second, we investigated whether the source of protein affects protein turnover. It was postulated recently that the source of protein as well as the amount of nitrogen in food could influence the metabolic response to feeding (8–10). Because our previous studies showed that elderly women especially had low protein turnover rates, the present study was conducted in elderly women to investigate in more detail the underlying mechanism behind this blunted response.

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SUBJECTS AND METHODS

Subjects

Twelve healthy, elderly women [69 ± 3.6 y of age, 1.58 ± 0.05 cm in height, weighing 69.3 ± 7.37 kg, with body mass index (BMI; in kg/m^2) of 28.1 ± 2.63] took part in the study. Subjects were recruited with advertisements in local media and through contacts with alliances for the elderly. All subjects were certified to be in good health by a staff physician on the basis of a physical examination and results of urine and blood tests. Subjects gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the Maastricht University's Ethics Committee.

Study design and diet composition

For the purpose of the study, 3 diets were prepared that had 3 similar energy contents (7.5, 8.25, and 9.0 MJ/d). All subjects followed either 2 or 3 diets for a period ≥2 wk. An interval ≥2 wk separated sequential diets. In diet A, 10.3% of total energy was provided by protein (5.3% animal, 5.0% vegetable), in diets B and C ≈20% of total energy was provided by protein (diet B: 14.5% animal, 5.1% vegetable; diet C: 5.0% animal, 15.1% vegetable). Subjects were fed according to their estimated energy intake based on a dietary questionnaire (11). Diet composition and the food products used are given in **Table 1**. During the intervention, all meals were provided at home. The subjects were not allowed to drink anything except water, tea, or coffee. Measurements were performed in 8 subjects after they consumed diet A, in 10 subjects after they consumed diet B, and in 10 subjects after they consumed diet C. This resulted in 6 paired measurements that permitted the comparison of diet A with diet B and diet C, and in 8 paired measurements that permitted the comparison of diet B with diet C. Mean daily energy intake was 123.5 ± 12.35 kJ/kg during diet A compared with 116.3 ± 12.06 kJ/kg during diet B (n = 6); when comparing diet A with diet C, mean energy intake was 117.4 ± 8.6 and 113.0 ± 9.7 kJ/kg, respectively (n = 6). Mean energy intake was 109.7 ± 12.15 kJ/kg during diet B compared with 111.0 ± 12.01 kJ/kg during diet C (n = 8).

Nitrogen balance

To determine nitrogen balance (calculated as nitrogen intake minus nitrogen in feces and urine), the subjects collected 24-h urine samples for 2 d and total feces for 3 d (during the last week of each diet period). Feces and urine were collected at home and all samples were brought to the institute at the end of each day of the metabolic balance study. Feces were collected for 3 d because this was the maximum compliance period (self-reported) for these elderly subjects. No fecal marker was used.

After total volume and total weight of urine and feces were measured, the nitrogen contents of both were measured with a Heraeus analyzer (type CHN-O-rapid; Hanau, Germany). Corrections were made for the remaining obligatory losses (8 mg N/kg body wt) (12).

TABLE 1
Diet composition

	Diet A	Diet B	Diet C
Protein (% of energy)			
Animal	5.3	14.5	5.0
Vegetable	5.0	5.1	15.2
Fat (% of energy)	35.8	28.2	30.8
Carbohydrate (% of energy)	53.9	52.1	49.1
Food items	Whole-meal bread[1–3]	Whole-meal bread[1–3]	Whole-meal bread[1–3]
	Margarine[1–3]	Low-fat margarine[1–3]	Low-fat margarine[1–3]
	Jam[1–3]	Jam[1,2]	Jam[1,2]
	Fruit sprinkles[1–3]	Cheese spread (20% fat)[1,2]	Cheese spread (20% fat)[1–3]
	Luncheon meat[1–3]	Luncheon meat[3]	Vegetable protein powder[1–3]
	Chocolate sprinkles[1–3]	Chocolate sprinkles[1]	Peanut butter[1–3]
		Cheese (40% fat)[1–3]	
		Cooked ham[2,3]	
		Chicken breast[2,3]	Chicken breast[1–3]
	Orange juice[1–3]	Orange juice[1–3]	Orange juice[1–3]
	Soft drink[1–3]	Soft drink[1–3]	Soy pudding[1–3]
	Powdered milk[1–3]	Evaporated milk[1–3]	Evaporated milk[2,3]
		Low-fat milk[1–3]	Low-fat milk[1–3]
	Biscuit[1–3]	Biscuit[2,3]	Biscuit with currants[1–3]
	Milk chocolate[2,3]	Milk chocolate[2,3]	Soymilk[2,3]
	Toast[3]	Toast[2]	
	Cake[3]	Candy bar[1–3]	Candy bar[3]
	Potato crisps[1]		
	Cookies[1–3]		
		Meat-based instant soup[1,2]	Vegetable-based instant soup[1]
	Frozen dinner[1–3]	Frozen dinner[1–3]	Frozen dinner[1–3]
	Low-fat fruit yogurt[3]	Low-fat fruit yogurt[1–3]	Low-fat fruit yogurt[1–3]

[1] For group receiving 7.50 MJ.
[2] For group receiving 8.25 MJ.
[3] For group receiving 9.00 MJ.
[4] The frozen dinners consisted of potatoes and vegetables in diets A, B, and C as well as meat in diets A and B, and soy products in diet C.

TABLE 2
Meal composition of diets

	Diet A	Diet B	Diet C
Protein (% of energy)			
Animal	5.4	15.1	4.7
Vegetable	5.2	4.9	16.0
Fat (% of energy)	34.5	30.0	31.5
Carbohydrate (% of energy)	55.0	50.0	47.7
Food items	Bread	Bread	Bread
	Margarine	Margarine	Margarine
	Meat	Cheese	Cheese
	Marmalade	Meat	Peanut butter
	Orange juice	Soft drink	Soymilk
	Yogurt	Yogurt	Protein powder

TABLE 3
Amino acid profile of the meals given during the L-[1-^{13}C]leucine infusion[1]

	Diet A	Diet B	Diet C	Requirement/d[2]
	$mg \cdot kg^{-1} \cdot h^{-1}$	$mg \cdot kg^{-1} \cdot h^{-1}$	$mg \cdot kg^{-1} \cdot h^{-1}$	*mg/kg*
Isoleucine	2.6	5.6	4.4	10
Leucine	5.1	10.9	8.6	14
Lysine	3.2	7.7	5.3	12
Methionine	1.2	3.0	1.9	13
Phenylalanine	3.1	6.1	5.6	14
Threonine	2.3	4.8	3.6	7
Tryptophan	0.7	1.4	1.2	3.5
Valine	3.2	6.9	5.5	10
Histidine	1.8	3.6	2.8	10
SEA	23.2	49.9	38.8	95
STA	69.6	131.6	114.7	—
EA:TA	5.0	5.4	5.2	—

[1] SEA, sum of essential amino acids; STA, sum of total amino acids.
[2] From reference 12.

Protein turnover as measured with L-[1-^{13}C]leucine

Whole-body protein turnover was measured with an 8-h primed, continuous infusion of L-[1-^{13}C]leucine (Cambridge Isotope Laboratories, Boston) on the last day of each study period. Each subject fasted for 12 h overnight and during the first 4 h of the infusion study. The L-[1-^{13}C]leucine studies were conducted in a laboratory at our institute. Before the start of the study, a venous catheter was inserted with aseptic technique into an antecubital vein of each subject's arm. One catheter was used for withdrawing blood samples. A priming dose of $NaH^{13}CO_3$ (0.085 mg/kg) and L-[1-^{13}C]leucine (3.8 μmol/kg) was administered into the other catheter. At 0800, the infusion of L-[1-^{13}C]leucine (3.6 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) was started at a constant rate (50 mL/h) for 8 h by using a calibrated pump (model 561; IVAC, San Diego). The first 4 h of the study were conducted with subjects in the fasted state, whereas for the last 4 h they were in the fed state. Compositions of and products used for the study meals were identical to the diet consumed the previous 2 wk (**Table 2**). Amino acid profiles of the diets are given in **Table 3**. Subjects received one-third of their daily energy intake in 9 equal portions. Subjects started with 2 portions at 1200 and received the other portions every 30 min during the 4-h fed period. Diet composition, as given during the protein turnover study, is given in Table 2. Blood and expired air were obtained just before the administration of the isotope and at 30-min intervals thereafter during the first, second, fifth, and sixth hours. During the third, fourth, seventh, and eighth hours, blood and expired air samples were collected at 15-min intervals. Each blood sample was collected into a syringe with heparin and transferred immediately to a tube that was kept on ice. Blood samples were separated and plasma was stored at −20 °C until analyzed. Plasma ^{13}C enrichment was measured with a gas chromatograph–mass spectrometer (as specified below). Expired air samples were obtained by having the subject breathe normally for 3 min into a 6.75-L bag. After 3 min, a 20-mL evacuated container was filled with a sample of the expired air. $^{13}CO_2$ enrichment in the expired air was measured by gas chromatography–infrared mass spectroscopy (Finnigan, Bremen, Germany). At 3, 4, 7, and 8 h after the start of the infusion, each subject's carbon dioxide production rate was determined by means of the ventilated hood technique (Oxycon β; Mijnhard, Bunnik, Netherlands).

Determination of plasma α-ketoisocaproic acid enrichment

^{13}C enrichment of intracellular leucine is based on measurement of the isotopic enrichment of the precursor in plasma, namely α-ketoisocaproic acid (KIC; 13). The isotopic analysis of [^{13}C]KIC was as follows (14): 50 μL internal standard (α-ketovaleric acid) was added to 200 μL plasma. This was deproteinized with 1 mL ethanol and vortex mixed. The solution was then centrifuged for 20 min (1250 × *g*, 4 °C). The supernate was transferred to a 13 × 100 mm screw-top tube and evaporated to dryness under nitrogen (50 °C) and dissolved in 200 μL distilled water plus 200 μL phenylenediamine (0.2 g/10 mL 4 mol HCl/L) and heated to 90 °C for 1 h. The solution containing the partially derivatized KIC was extracted twice with 1 mL ethyl acetate (centrifuged each time for 10 min, 1500 × *g*, 4 °C). The ethyl

TABLE 4
Nitrogen balance[1]

	Diet A compared with diet B (n = 6)		Diet A compared with diet C (n = 6)		Diet B compared with diet C (n = 8)	
	Diet A	Diet B	Diet A	Diet C	Diet B	Diet C
	$mg \cdot kg^{-1} \cdot d^{-1}$					
Nitrogen intake	125.2 ± 10.6[2]	201.5 ± 20.9	120.7 ± 11.2[2]	209.3 ± 16.3	190.0 ± 20.4	206.7 ± 22.8
Nitrogen in feces	33.6 ± 21.8	36.6 ± 16.2	36.0 ± 20.4	36.8 ± 14.3	31.4 ± 16.0	29.6 ± 13.6
Nitrogen in urine	125.1 ± 13.3[2]	155.6 ± 49.1	115.7 ± 10.3[2]	163.9 ± 30.5	143.7 ± 32.3	147.0 ± 34.7
Nitrogen balance[3]	−41.5 ± 15.8[2,4]	1.6 ± 50.9	−39.1 ± 15.7[2,4]	0.6 ± 34.9	7.0 ± 42.5	22.1 ± 32.4

[1] $\bar{x}$ ± SD.
[2] Significantly different from other diet in pairing, $P < 0.05$.
[3] Corrections were made for obligatory losses (8 mg N/kg body wt; 12).
[4] Nitrogen balance was negative with diet A, but balanced with diets B and C.

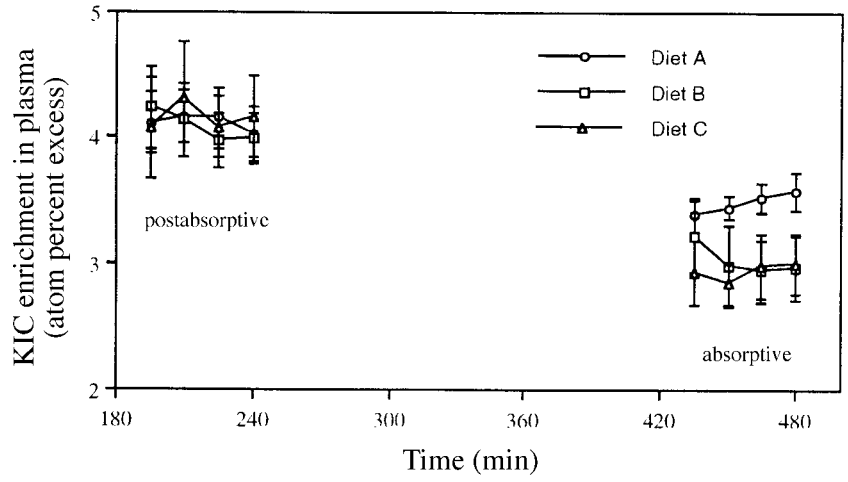

FIGURE 1. Mean (±SEM) plasma α-ketoisocaproic acid (KIC) enrichment values measured at steady state in the postabsorptive and absorptive state during diets A, B, and C. Diet A: 5.3% of energy intake as animal protein and 5% as vegetable protein; diet B: 14.5% of energy as animal protein and 5.1% as vegetable protein; diet C: 5.0% of energy as animal protein and 15.1% as vegetable protein.

acetate fraction was combined in a 5-mL Pierce vial (Oud Beijerland, Netherlands) and evaporated to dryness under nitrogen (21°C). The residue was taken up in 50 μL pyridine and 50 μL *N*,*O*,-bis(trimethyl-silyl)trifluoroacetamide with 1% trimethylchlorosilane. After this, the plasma [^{13}C]KIC isotope enrichment was measured on a gas chromatograph–mass spectrometer (INCOS XL GC/MS Varian 3400 GC with 1075 split/splitless injector; Finnigan).

Calculations of the rate of protein turnover

The rate of protein turnover was calculated as described previously (13). In short, leucine turnover was measured from the dilution of L-[1-^{13}C]leucine infusion in plasma leucine at isotopic steady state: $Q = i[(E_i/E_p) - 1]$; where i is the L-[1-^{13}C]leucine infusion rate (mmol · kg^{-1} · h^{-1}), E_i is the enrichment of the L-[1-^{13}C]leucine infused (atom percent excess), and E_p is the [^{13}C]KIC enrichment in the plasma at steady state (atom percent excess). The rate of leucine oxidation is $O = F^{13}CO_2\,[(1/E_p) - (1/E_i)] \times 100$. Where $F^{13}CO_2$ is the rate of $^{13}CO_2$ released by leucine tracer oxidation (mmol ^{13}C · kg^{-1} · h^{-1}). From these calculations, the rate of leucine incorporation into protein is calculated with $Q = S + O = B + I$; $S = Q - O$. The leucine parameters mentioned above were converted to corresponding estimates of whole-body protein turnover by multiplying the leucine values by the constant (24 h/d)/(590 μmol leucine/g protein) to give values of g protein · kg^{-1} · d^{-1}.

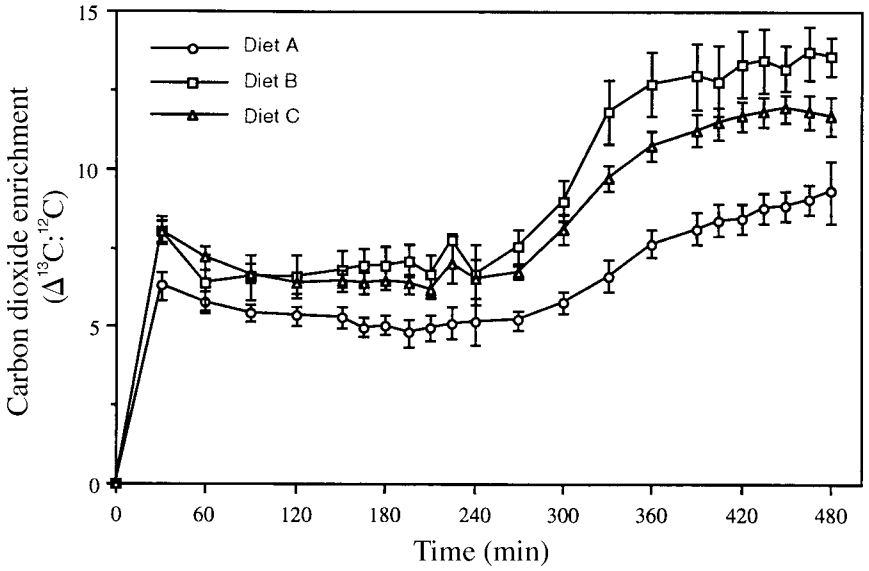

FIGURE 2. Mean (±SEM) carbon dioxide enrichment measured in expired air during the L-[1-^{13}C]leucine infusion experiment. Steady state data in the postabsorptive and absorptive state during diets A, B, and C were used for the calculations. Diet A: 5.3% of energy intake as animal protein and 5% as vegetable protein; diet B: 14.5% of energy as animal protein and 5.1% as vegetable protein; diet C: 5.0% of energy as animal protein and 15.1% as vegetable protein.

TABLE 5
Protein metabolism[1]

	Diet A compared with diet B (n = 6)		Diet A compared with diet C (n = 6)		Diet B compared with diet C (n = 8)	
	Diet A	Diet B	Diet A	Diet C	Diet B	Diet C
	$g \cdot kg^{-1} \cdot d^{-1}$					
Q						
Fasted	3.41 ± 0.59[2]	3.63 ± 0.26[2]	3.52 ± 0.65[2]	3.51 ± 0.64[2]	3.50 ± 0.37[2]	3.59 ± 1.37[2]
Fed	4.00 ± 0.52[3]	5.13 ± 0.68	4.02 ± 0.53	4.48 ± 1.15	4.71 ± 0.44	4.69 ± 1.40
O						
Fasted	0.36 ± 0.08[2,3]	0.49 ± 0.12	0.34 ± 0.06[2,3]	0.45 ± 0.04[2]	0.46 ± 0.06[2]	0.47 ± 0.19[2]
Fed	0.93 ± 0.15[3]	1.80 ± 0.26	0.96 ± 0.15[*]	1.56 ± 0.29	1.65 ± 0.15	1.48 ± 0.26
S						
Fasted	3.05 ± 0.53	3.14 ± 0.22	3.17 ± 0.61	3.06 ± 0.63	3.05 ± 0.36	3.12 ± 1.20
Fed	3.07 ± 0.42[3]	3.33 ± 0.49	3.06 ± 0.43	2.93 ± 0.94	3.06 ± 0.40	3.21 ± 1.23
B						
Fasted	3.41 ± 0.59[2]	3.63 ± 0.28[2]	3.51 ± 0.65[2]	3.52 ± 0.64[2]	3.50 ± 0.37[2]	3.59 ± 1.37[2]
Fed	2.37 ± 0.56[3]	1.51 ± 0.80	2.44 ± 0.64	1.90 ± 1.20	1.16 ± 0.54	2.17 ± 1.38
S−*B*						
Fasted	−0.36 ± 0.08[2,3]	−0.48 ± 0.12[2]	−0.34 ± 0.06[2,3]	−0.45 ± 0.04[2]	−0.46 ± 0.06[2]	−0.47 ± 0.19[2]
Fed	0.69 ± 0.26[3]	1.82 ± 0.46	0.62 ± 0.26[3]	1.04 ± 0.46	1.90 ± 0.32[3]	1.04 ± 0.32

[1] $\bar{x}$ ± SD. *Q*, protein flux; *O*, protein oxidation; *S*, protein synthesis; *B*, protein breakdown; *S*−*B*, difference between protein synthesis and protein breakdown.
[2] Significantly different from fed, $P < 0.05$.
[3] Significantly different from other diet in pairing, $P < 0.05$.

Statistical analyses

When comparisons were made between diets, a nonparametric test (Wilcoxon signed-rank test) was used (STATVIEW 512+; Abacus Concepts, Berkeley, CA). Data are given as means ± SDs. Because not all subjects were measured with all diets, separate tables were made for the comparison of diet A with diets B and C and for the comparison of diet B with diet C.

RESULTS

Nitrogen balance

Nitrogen balance results are given in **Table 4**. Nitrogen intake was higher during diets B and C than during diet A. There was no effect of the protein content of the diet on the nitrogen excretion in the feces. Urinary nitrogen excretion was higher during the high-protein diets (B and C). Subjects were in negative nitrogen balance while consuming diet A, whereas subjects were in nitrogen balance when consuming diets B and C. Moreover, there were differences in nitrogen balance between the diets; nitrogen balance was more positive during the higher protein intake (diets B and C) as compared with diet A.

Protein turnover

Protein turnover data were based on steady state enrichment of breath and plasma samples (the CV of the 4 samples taken in the last hour of the postabsorptive and the absorptive phase of the L-[1-^{13}C]leucine infusion experiment was <5%). The KIC

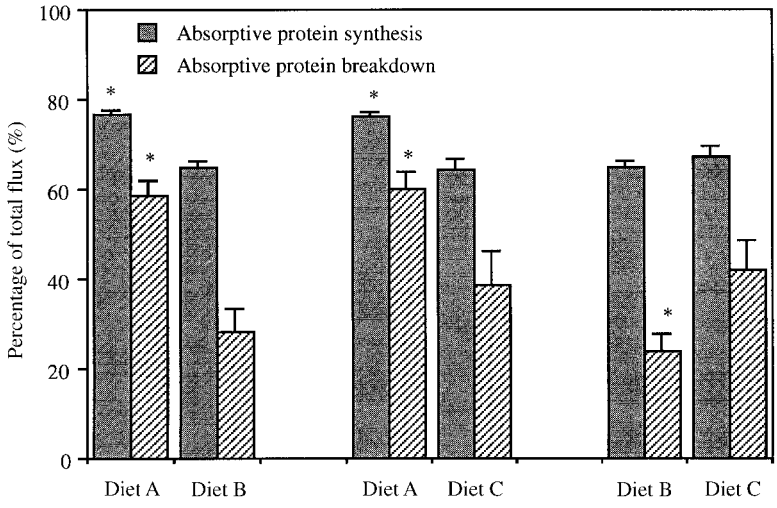

FIGURE 3. Absorptive protein synthesis and breakdown data as a percentage of total flux. [*]Significantly different from other diet in the pairing, $P < 0.05$. Diet A: 5.3% of energy intake as animal protein and 5% as vegetable protein; diet B: 14.5% of energy as animal protein and 5.1% as vegetable protein; diet C: 5.0% of energy as animal protein and 15.1% as vegetable protein).

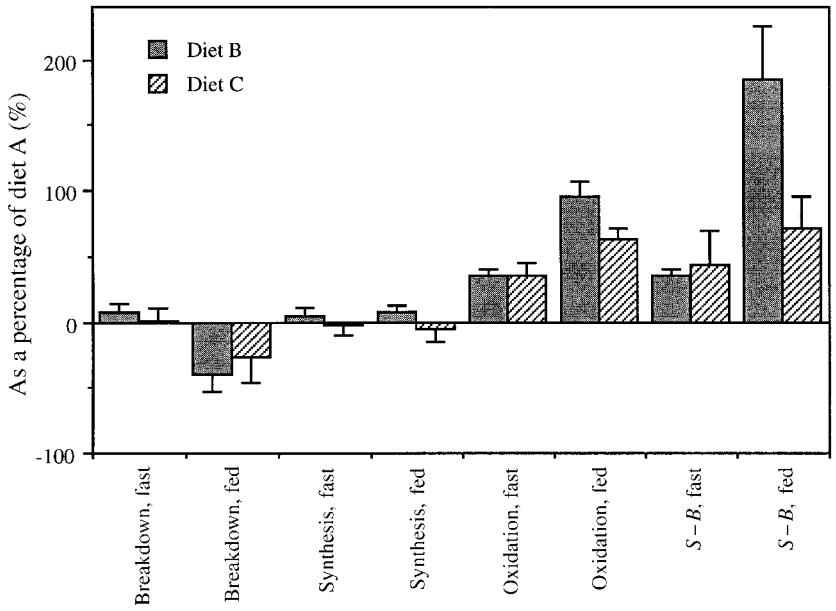

FIGURE 4. Postabsorptive protein synthesis, breakdown, oxidation, and net protein breakdown and synthesis data during diet B or C expressed as a percentage of the same parameters during diet A. Diet A: 5.3% of energy intake as animal protein and 5% as vegetable protein; diet B: 14.5% of energy as animal protein and 5.1% as vegetable protein; diet C: 5.0% of energy as animal protein and 15.1% as vegetable protein. $S - B$ is the difference between protein synthesis and protein breakdown.

enrichment data are presented in **Figure 1** and the carbon dioxide enrichment in the breath samples is given in **Figure 2**. When protein turnover results during diets A and B were compared (**Table 5**), it was clear that protein flux, protein breakdown (*B*), and protein synthesis (*S*) were higher during the fed state of diet B. Protein oxidation was also higher during diet B than during diet A in both the fasted and fed states. Net protein breakdown ($S - B$ during fasting) and net protein synthesis ($S - B$) were higher during diet B than during diet A.

When protein turnover results during diets A and C were compared (Table 5), we noted that protein oxidation was higher during diet C than during diet A in the fasted state as well as in the fed state. There was no effect of the protein content of the diet on protein flux, protein breakdown, or protein synthesis. Net protein breakdown and net protein synthesis were higher during diet C than during diet A. When protein turnover results during diets B and C were compared (Table 5), no differences were seen in protein flux, protein oxidation, or protein synthesis. Although not significantly so, protein breakdown tended to be higher with diet C than with diet B during feeding ($P = 0.08$). Furthermore, net protein synthesis was higher during diet B. When results during fasting were compared with those during feeding (Table 5), we observed that with all diets (A, B, and C) there was an increase in protein flux and protein oxidation. Protein breakdown decreased during feeding with all diets, whereas there was no effect on protein synthesis.

Absorptive protein breakdown and synthesis data are also expressed as a percentage of total flux (**Figure 3**). Expressed in this way, *S* constitutes ≈76% of the total flux in diet A (diet A compared with diet B: 76.6 ± 0.9%; diet A compared with diet C: 76.1 ± 1.0%), 64% in diet B (diet B compared with diet A: 64.8 ± 1.2%; diet B compared with diet C: 64.8 ± 1.3%), and ≈65% in diet C (diet C compared with diet A: 64.5 ± 2.2%; diet C compared with diet B: 67.1 ± 2.3%). Protein breakdown constitutes ≈58% of the total flux in diet A (diet A compared with diet B: 58.6 ± 3.3%; diet A compared with diet C: 59.8 ± 3.8%), ≈26% in diet B (diet B compared with diet A: 28.1 ± 5.4%; diet B compared with diet C: 23.9 ± 3.9%), and ≈40% in diet C (diet C compared with diet A: 38.5 ± 7.8%; diet C compared with diet B: 41.9 ± 6.5%).

The relative increases or decreases in protein oxidation, synthesis, and breakdown are presented in **Figure 4**. Protein breakdown was suppressed by 40 ± 13% when changing from diet A to diet B and by 27% when changing from diet A to diet C. Postabsorptive protein synthesis increased 9 ± 4% when changing from diet A to diet B and decreased by 5 ± 11% when changing from diet A to diet C. Postabsorptive protein oxidation increased by 95 ± 12% when changing from diet A to diet B, and by 63 ± 9% when changing from diet A to diet C.

DISCUSSION

At the end of each dietary period, nitrogen balance was determined over 3 d. There was no effect of the protein content of the diet on the nitrogen excretion in feces, whereas urinary nitrogen excretion was higher during the high-protein diets (B and C). As described earlier (24), subjects were in negative nitrogen balance when consuming the recommended intake of 0.8 g protein/kg body wt, whereas subjects were in nitrogen balance when consuming diets B and C. These results indicate that a protein intake of 0.8 g/kg body wt is close to the daily requirement. However, nitrogen balance measurements reflect only the balance between overall body protein synthesis and breakdown rates and nitrogen balance may be achieved within a wide range of protein turnover rates (15).

Looking at the fasting and feeding results within one diet, it can be concluded that the same changes were seen in all 3 diets. Whole-body protein flux and protein oxidation increased when

changing from fasting to feeding. Protein synthesis was not affected by feeding, whereas protein breakdown decreased during feeding. All subjects were in net protein synthesis during the absorptive part of the study during all diets. In elderly women, changes in protein metabolism when changing from the postabsorptive to the absorptive state (within one diet) were the same as reported before in young adults (16–18): the main effect of feeding was a reduction in protein breakdown, with very little change in synthesis. As described earlier in young adults (19–21), the magnitude of the changes was dependent on protein quantity. However, the present study revealed that the protein source is also important.

Only 4 subjects completed all 3 diets. Statistical analyses with only 4 subjects revealed that none of the measured parameters changed significantly as a result of a change in dietary protein intake. Therefore, separate comparisons were made between the different diets. Comparison of data on postabsorptive protein metabolism during diets A, B, C showed that there were no significant differences in protein flux, synthesis, or breakdown between the diets. However, fasting protein oxidation and net protein breakdown were higher with the higher-protein diets (B and C) than with diet A. No differences in fasting protein metabolism were found between the high-animal-protein diet and the high-vegetable-protein diet.

The effect of the protein source seemed to be more pronounced during feeding because when the high-animal-protein diet was fed, effects agreed with results reported before in young adults: the magnitude of the changes in protein metabolism during feeding was dependent on protein quantity (1, 19–22). With the high-animal-protein intake, the absorptive protein oxidation rate, flux, and synthesis were higher whereas absorptive protein breakdown was lower than for diet A. Furthermore, net protein synthesis was higher during diet B. Data on protein metabolism during the 10%-protein diet (A) and the high-vegetable-protein diet (C) showed a similar effect on protein oxidation and net protein synthesis, which were both higher during diet C. However, absorptive protein flux and absorptive protein synthesis were not affected by the amount of vegetable protein in the diet. Remarkably, absorptive protein breakdown values were not lower with the high-vegetable-protein diet than with the 10%-protein diet.

Finally, comparisons were made between the high-animal- and the high-vegetable-protein diets. The protein source had no effect on protein flux, protein oxidation, or protein synthesis during feeding. However, protein breakdown during feeding tended to be lower during the high-animal-protein diet ($P = 0.08$), indicating that while consuming the high-vegetable-protein diet, protein breakdown was not inhibited to the same extent as during the high-animal-protein diet. A significant difference was seen in net protein synthesis, which was higher while consuming the high-animal-protein diet. The lack of significance may have been due to the limited power of this study. Therefore, absorptive protein breakdown and synthesis data are also expressed as a percentage of total flux (Figure 3). Expressed in this way, synthesis constituted ≈76% of the total flux in diet A, 64% in diet B, and ≈65% in diet C. However, protein breakdown constituted ≈58% of the total flux in diet A, ≈26% in diet B, and ≈40% in diet C. Thus, the source of dietary protein suppressed protein breakdown over and above that of dietary quantity alone. The relative increase or decrease in protein oxidation, synthesis, and breakdown was also calculated and is presented in Figure 4. The data show that absorptive protein breakdown was suppressed by 40 ± 13% when the subjects changed from diet A to diet B, and by 27% when they changed from diet A to diet C. On the other hand, postabsorptive protein synthesis increased 9 ± 4% when subjects changed from diet A to diet B and decreased by 5 ± 11% when they changed from diet A to diet C. Finally, postabsorptive protein oxidation increased by 95 ± 12% when subjects changed from diet A to diet B and by 63 ± 9% when they changed from diet A to diet C. These observations suggest a difference in the intensity of the adaptive response to protein diets of animal and vegetable origin. The magnitude of the response to diet B can be explained by a greater proportional change in protein synthesis and breakdown, whereas diet C had an effect only on protein breakdown.

Data on the differences between the 10%-protein diet and the high-animal-protein diet agree with the theory of the labile protein pool: despite the large intake of amino acids during a protein meal, the peripheral plasma concentrations of the essential amino acids are only modestly perturbed. It has been suggested that the stability of the amino acid pool during meals is achieved by an increase in protein synthesis, an inhibition of protein breakdown (less endogenous amino acids are released into the free amino acid pool), and by an increased rate of amino acid oxidation (probably acting as an overflow mechanism). The increased protein stores associated with protein meals are called labile protein stores (22, 23). These pools could be located in the gut (9), the liver, or other parts of the body. This labile protein pool could play a significant role in the postabsorptive phase by providing essential amino acids to the body free amino acid pool. It has been speculated that vegetable proteins are less capable of suppressing protein breakdown and stimulating protein synthesis. In other words, vegetable proteins do not seem to expand the labile protein pool to the same extent as do animal proteins. Interpreted in this way, our results agree with the findings of Deutz and Soeters (9), who observed that in pigs, soy-based protein meals induced a lower gut protein synthesis rate than did casein-based protein meals.

It is difficult to explain the different response in protein metabolism to the high-animal- and high-vegetable-protein diets and one can only speculate about the possible causes. First, the observed differences may be explained by differences in the amino acid compositions of the diets. As expected, and shown in Table 3, the amino acid composition did differ between the low- and the high-protein diets. However, there were also slight differences in the amount of essential amino acids in the high-animal-protein diet and the high-vegetable-protein diet. It is not likely that these differences account fully for the differences in protein turnover during feeding because the essential amino acid concentration of the high-vegetable-protein diet was higher than that of diet A whereas there were no differences in protein breakdown or synthesis during these 2 diets. Furthermore, it was shown that the response of leucine kinetics to feeding is modified by the molecular form of nitrogen intake, with oligopeptides inducing a higher oxidation and a higher protein synthesis and a lesser inhibition of protein breakdown (8). We cannot exclude the possibility that the molecular form of the nitrogen intake explains the differences in response to the high-animal- and the high-vegetable-protein diet. Finally, a minor weight loss was observed during all diet periods. Mean weight loss was 0.50 ± 0.41 kg/14 d during diet A and 0.63 ± 0.47 kg/14 d during diet B ($n = 6$). When diet A was compared with diet C, mean weight loss was 0.50 ± 0.61 kg/14 d and 0.47 ± 0.70 kg/14 d,

respectively (n = 6). Mean weight loss was 0.76 ± 0.45 kg/14 d during diet B compared with 0.46 ± 0.34 kg/14 d during diet C (n = 8). However, these minor weight losses were not significantly different between the dietary interventions periods, indicating that differences in energy balance do not contribute to the differences seen on protein metabolism.

It is hard to speculate whether there are biological implications of the effect of the protein source on protein metabolism. On a 24-h basis, the results of the nitrogen balance study showed no effect of the protein source. However, protein turnover results obtained during a 2 × 4 h study did show a tendency toward differences in protein breakdown and a difference in net protein synthesis in favor of a high-animal-protein diet. In summary, we conclude that subjects were in nitrogen balance during diets B and C, whereas protein turnover data indeed showed some differences. The immediate result of food absorption is the depression of whole-body protein breakdown, and the magnitude of this effect is dependent on both the protein quantity and quality. There is relatively little effect of protein quantity or quality on protein synthesis. The decrease in protein degradation that is normally observed during the fed state is less pronounced when the diet is rich in vegetable protein than for an equivalent amount of animal protein.

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